

## Review Article

# Survey of Canine *Dirofilaria immitis* Infection in New Caledonia

S. Watier-Grillot,<sup>1,2</sup> J.-L. Marié,<sup>1,2</sup> O. Cabre,<sup>3,4</sup> and B. Davoust<sup>1,2</sup>

<sup>1</sup> Direction Régionale du Service de Santé des Armées de Toulon, Service Vétérinaire des Armées, BP 860, 83800 Toulon Cedex 9, France

<sup>2</sup> Groupe de Travail en Epidémiologie Animale (GTEA) du Service de Santé des Armées, Secteur Vétérinaire de Marseille, BP 30182, 13276 Marseille Cedex 9, France

<sup>3</sup> Conseiller Technique Vétérinaire auprès de la Direction Interarmées du Service de Santé des Forces Françaises en Nouvelle Calédonie, BP 2973, 98846 Nouméa Cedex, France

<sup>4</sup> Secteur Vétérinaire de Lyon, 9 rue du Mas Rillier, 69142 Rillieux-la-Pape Cedex, France

Correspondence should be addressed to S. Watier-Grillot, gillinghamvet@yahoo.fr

Received 15 October 2010; Revised 17 December 2010; Accepted 8 February 2011

Academic Editor: Georg Duscher

Copyright © 2011 S. Watier-Grillot et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Canine dirofilariasis is a frequent parasitic disease in New-Caledonia. A survey of canine heartworm (*Dirofilaria immitis*) infection among dogs from the cities of Tontouta, Nandai and Nouméa, was performed in March 2009 using two antigen test kits; the microwell ELISA test: DiroCHE (Synbiotics Europe) and the Rapid Immuno Migration (RIM) test: WITNESS DIROFILARIA (Synbiotics Europe). Blood samples were collected from 64 dogs: 49 strays and 15 military working dogs. The military dogs received a permanent chemoprophylaxis (moxidectin). In 11 stray dogs, both tests were positive (22.4%). All the military dogs were negative, showing efficiency of chemoprophylaxis. Results were discrepant in 6 dogs, negative with one test and doubtful with the other. Antigen heartworm test kits are available and reliable diagnostic tools. They are useful to evaluate the efficiency of chemoprophylaxis and to detect infected animals in order to treat them and to prevent the spreading of the disease.

## 1. Introduction

*Dirofilaria immitis* is the causative agent of canine heartworm disease. Adult worms live in the pulmonary arteries where females produce embryos which are taken up by bloodsucking mosquitoes. These vectors transmit the infection to other animals. Dogs are the main hosts; however, the parasite can also infect cats, ferrets, and other mammals such as the red fox and the coyote. The zoonotic risk is also important. In dogs, the infection is chronic and leads to congestive heart failure. The distribution of *Dirofilaria immitis* is worldwide, and the parasite has been found in tropical, subtropical and temperate regions [1].

Canine dirofilariasis is an endemic helminthosis in New Caledonia with a high prevalence of infection [2]. New Caledonia is located around 21°30'S 165°30'E/21.5°S 165.5°E, approximately, 1200 kilometres east of Australia and 1500 kilometres northwest of New Zealand, in the subregion of Melanesia, southwest Pacific. It comprises

a main island (*Grande Terre*), the Loyalty Islands, and several smaller islands. The capital and largest city of the territory is Nouméa. According to a previous survey [3] in 1993, estimated prevalence of canine dirofilariasis was 57 ( $\pm 8$ )%. Two epidemiologic situations were distinguished between strays and dogs owned by people, with prevalences of 66% and 38%, respectively.

Because of the importance of heartworm disease in veterinary medicine and public health, it is necessary to carry out a diagnosis by efficient and well-adapted methods. In infected dogs, a confirmed diagnosis of heartworm disease associated with an efficient clinic examination allows treatment before symptoms of cardiac failure appear. Uninfected dogs can be protected through efficient prophylactic measures. Diagnosis and screening of the disease are essential for the control of the disease because they permit identification of the sources and reservoir hosts of the parasite, thus facilitating prevention of the disease.

Heartworm antigen test kits are widely used in veterinary clinics to detect heartworm infection in dogs [1, 4–7]. These kits have largely supplanted the use of microfilaria tests both for screening and diagnosis, because of their high sensitivity and specificity and because of the antimicrofilarial effects of commonly used heartworm chemoprophylaxis using macrocyclic lactones [5, 8–12]. However, microfilaria testing is complementary and may be done in tandem with antigen testing to specifically determine whether this life-cycle stage is also present in dogs that are antigenemic. Even in areas where the prevalence of heartworm infection is high, many (about 20%) heartworm-infected dogs may not be microfilaremic, and this figure is even higher for dogs on a macrocyclic lactone prevention program [4].

## 2. Material and Methods

**2.1. Animals.** In March 2009, a survey for the detection of *D. immitis* infection was carried out in dogs from New Caledonia. Of a total of 64 dogs, 49 were local stray dogs and 15 were military working dogs. The military dogs came from the cities of Tontouta and Nandaï and the stray dogs from Nouméa. Tontouta and Nouméa are separated by 42 kilometres away, whilst Nandaï and Nouméa are 190 kilometres away from each other. Breed, sex, and age of stray dogs were varied. Military dogs were male Belgium shepherds with an average age of five years.

Military working dogs spend mission time ranging from several months to several years in New Caledonia. During this period, they receive a year-round chemoprophylaxis, based on the administration of moxidectin (sustained release) every 6 months: ProHeart 6 (Pfizer Santé animale, Paris, France) by subcutaneous injection of 0.05 mL of the suspension/kg body weight. This provides 0.17 mg/kg body weight. This dosage is reported as being effective in preventing the disease in dogs [1, 4, 7]. No data are available about the sanitary status or possible chemoprophylaxis of the stray dogs. Nevertheless, as they are feral animals, we suppose that they did not receive any chemoprophylaxis. No symptoms of heartworm disease were identified in any of the dogs included in the study.

**2.2. Laboratory Diagnostics.** To evaluate the prevalence of heartworm infection in dogs, two antigen test kits were used in a blind study using serum drawn from 59 stray dogs from the area. The efficiency of chemoprophylaxis was assessed on 15 military working dogs. The test kits included the microwell ELISA test: DiroCHEK (Synbiotics Europe) and the rapid immunomigration (RIM) test: WITNESS DIROFILARIA (Synbiotics Europe).

All test kits were used prior to their expiration date and were stored and used as recommended by the manufacturer. For each dog, two samples of serum were collected. All sixty-four samples were sequentially tested with each test kit. Technical reasons prevented examination for microfilaria (Knott test) and necropsies could not be performed.

DiroCHEK is an enzyme-linked immunoabsorbent assay (ELISA) for the detection of adult *D. immitis* antigen in

canine and feline plasma or serum. DiroCHEK is highly specific (specificity of 100%), sensitive (range of sensitivity of 85% to 100%), and easy to use (Synbiotics Corporation/Direction inserts for use of the DiroCHEK test). Test results can be obtained within 15 minutes. The reaction wells are coated with antibodies directed against *D. immitis* antigen. Another antibody is labelled with horseradish peroxidase. Any antigen present in plasma or serum is bound by the antibody and coated with the enzyme-linked antibody to form a specific complex. Any free enzyme-linked antibody is washed away, and a chromogenic substrate is added. In the absence of *D. immitis* antigen, no colour change will be observed. The development of a blue colour specifically indicates the presence of *D. immitis* antigen from heartworms.

The WITNESS DIROFILARIA test is easy to implement, when based on rapid immunomigration (RIM) technology (Synbiotics Corporation/Direction inserts for use of the WITNESS DIROFILARIA test). It is highly specific (specificity of 100%) [13] with values ranging from 71% to 95% [13]. The test uses antibodies directed against specific epitopes of a soluble antigen of *D. immitis*. The sample that contains this antigen is put into contact with sensitised gold particles. The resultant complex then migrates on the membrane before being caught in a reactive area, where complex concentration creates a strongly apparent pink-coloured band. A control band is located on the opposite side of the membrane to ensure that the test is performed properly.

Statistical comparison of the prevalence of infection between stray dogs and military dogs was conducted using a Khi-2 test.

## 3. Results

For 11 stray dogs, both tests were positive (22.4%). All the military working dogs were negative with both tests. However, results were discrepant for 6 dogs (5 strays and 1 military dog); for 3 dogs, results were negative with WITNESS DIROFILARIA test and doubtful with DiroCHEK; for 3 other dogs (including 1 military dog), results were doubtful with WITNESS DIROFILARIA and negative with DiroCHEK. Samples were positive with both tests, suggesting a good correlation and consistency (100%) between the two tests. Infection rates between stray and military dogs were significantly different (Khi-2 (Yates correction) = 5.46;  $P < .04$ ).

## 4. Discussion

Since microfilaremia and necropsy studies were not performed, it is not possible to correlate the results of the antigen test kits with the adult heartworm infection status, that is, infected versus uninfected dogs. If the missing data were available, it would have been interesting to compare the performance between the WITNESS DIROFILARIA and the DiroCHEK tests. Moreover, such data would have permitted the evaluation of the limits of performance of the tests, and

to interpret the results with respect to heartworm burdens, as test kits are less sensitive with low heartworm burdens [5, 7–9, 13].

All positive samples were positive with both tests, suggesting good correlation and consistency (100%) between them.

In the absence of a diagnosis for microfilaria, the interpretation issues for positive antigen tests are as follows [6]:

- (i) heartworm infection (positive microfilaria diagnosis);
- (ii) heartworm occult infection (negative microfilaria diagnosis), with various possibilities: unisex infection (female worms); immune-mediated clearance of microfilaria; after monthly chemoprophylaxis or after microfilaricide treatment; infection by adults (5–6 months after infection).

Without microfilaria diagnosis, the interpretation issues for negative antigen tests are as follows [6]:

- (i) absence of heartworm infection (negative microfilaria diagnosis);
- (ii) heartworm infection (positive microfilaria diagnosis), with various possibilities; microfilaria are not *D. immitis*; absence or undetectably low levels of *D. immitis* antigen; immune clearance of antigen-antibody complexes; adults dead and antigen cleared, but microfilaria still present; microfilarial contamination of lysing solution, dye, or filter chamber; transfused with microfilaremic blood; prenatal transfer of microfilariae; antigen destroyed due to improper storage or treatment of sample.

Significant differences in sensitivity, accuracy, and predictive values among test kits in the present study can explain discrepant results.

Tests using ELISA technology (including DiroCHEK) are more sensitive than tests using lateral flow immunoassay methodology (including WITNESS DIROFILARIA) [7–9]. Contrary to ELISA technology, rapid immunomigration (RIM) does not include a washing stage of the matrix, bidirectional flow, and response amplification. These three characteristics can explain the better sensitivity of ELISA tests. Results of a previous study [8] indicated that sensitivity values ranged from 78% to 84%, respectively, with immunochromatographic and ELISA test formats.

The accuracy and negative predictive value of tests using ELISA (including DiroCHEK) are significantly higher than that of tests using rapid immunochromatographic methodology (including WITNESS DIROFILARIA) [8].

Our results show the efficiency of chemoprophylaxis (using moxidectin) used on military working dogs. However, the newest tests (including WITNESS DIROFILARIA and DiroCHEK tests) are quite sensitive, and antigen from dead heartworms has been detected for up to 3 to 4 months (Synbiotics Corporation data). Therefore, for definitive diagnosis, in order to confirm the success of adulticide therapy, dogs have to be retested 5 and 9 months later. If

the test at 5 months is negative, testing at 9 months can be avoided [6].

Transmission of dirofilariasis is dependent upon the presence of sufficient numbers of infected, microfilaremic hosts and reservoirs, susceptible mosquitoes, and a suitable climate to permit extrinsic incubation of the parasite in the mosquito intermediate host [14]. A pivotal prerequisite for heartworm transmission is a climate that provides adequate temperature and humidity to support a viable mosquito population and sustains sufficient heat to allow maturation of ingested microfilariae to infective, first-stage larvae (L3) within the intermediate host. It has been shown under laboratory conditions in three mosquito species that maturation of larvae within mosquitoes ceases at temperature below 14 degrees Celsius, and similar activity is expected in other mosquitoes capable of transmitting heartworms [4]. The time required for the development of microfilariae to the infective stage in the mosquito is temperature dependent. At 27°C and 80% relative humidity, development is shorter and takes about 10–14 days [4].

The climate of New Caledonia is more or less tropical, with two seasons: September to March, warm and humid with temperatures averaging between 25 and 27 degrees Celsius; April to August, cool and dry with temperatures averaging between 20 and 23 degrees Celsius. The average yearly temperature is 23 degrees Celsius. The average humidity rate is 80%. New Caledonia flora is very rich and various. The three primary vegetations of the country are the lowland rain forests, the mountainous forests, and the wet maquis forests (2005–2011 World Health Organisation Regional Office for the Western Pacific). The geographical and climatic features, among other factors, contribute to the development of arthropod and insect vectors of pathogens, including mosquitoes. Except *Anopheles*, many mosquito kinds and species are recens in New Caledonia: *Aedes*, *Culex*, *Mansonia*, and *Psorophora* (2005–2011 World Health Organisation Regional Office for the Western Pacific). All species are competent for *D. immitis* transmission [15]. According to previous data, climate of New Caledonia also favours the transmission and the life cycle of the parasite.

New Caledonia fauna is not as rich as its flora (2005–2011 World Health Organisation Regional Office for the Western Pacific). Land animals of New Caledonia include indigenous mammals (flying foxes, bats). All the other mammals have been introduced (rusa deer; wild pigs; horses, cattle, and other stocks; pets: dogs and cats; rats and mice, etc.). Wildlife reservoirs play a role in perpetuation and transmission of the parasite *D. immitis* to dogs [16]. Moreover, many free-roaming (i.e., stray) dogs and cats are present in urban and rural areas in New Caledonia. These animals contribute to the distribution, transmission rate and prevalence of heartworm infection in the country. Indeed, free-roaming cats and dogs are at high risk of acquiring vector-borne pathogens, mainly because they are often untreated against ectoparasites, thus representing an easy feeding source for them. In addition, the general conditions of these animals (e.g., poor nutrition) may contribute to susceptibility to heartworm infection. Likewise, when infected, free-roaming

cats and dogs are often neither monitored nor treated against *D. immitis*.

Human heartworm infection is incidental and rare. Human cases have been reported mainly in areas of high canine prevalence [16], among which there is New Caledonia [17], highlighting the importance of heartworm testing and chemoprophylaxis in all dogs to reduce the risks of man infection.

Because 22.4% of stray dogs included in this study were positive for *D. immitis* adult antigen, dogs living in this area should be considered at high risk for developing heartworm infection, and they are likely to become infected with *D. immitis* if appropriate precautions are not taken.

An annual occult heartworm test alone is considered appropriate for dogs receiving year-round monthly treatments [1, 17]. If the chemoprophylactic treatment is performed with a sustained release preparation, periodic testing (each 2 or 3 years) will ensure that there have been no efficacy breaks [1]. Nevertheless, as the performance of antigen tests decreases in dogs with low heartworm burdens and/or near the distribution limits of the parasite [18], a more accurate diagnosis of infection could be obtained using a complementary test (particularly a concentration test such as the modified Knott test) in addition to an antigen test. An annual screening using both tests is also useful to assess and control the efficiency of the chemoprophylaxis carried out on the military dogs.

## 5. Conclusions

In March 2009, an original survey was carried out on dogs from New Caledonia, using two heartworm antigen test kits (DiroCHEK and WITNESS DIROFILARIA, Synbiotics).

In the city of Nouméa, the prevalence of canine heartworm infection was 22.4% (11/49) in our sample. In the nearby cities of Tontouta and Nandaï, all results were negative for 15 military working dogs. These results demonstrate the efficiency of chemoprophylaxis (with moxidectin) used on military working dogs.

Because microfilaremia examination and necropsy were not performed, the results can neither provide the prevalences of definitive heartworm infection, nor the whole data concerning performance of the tests.

In order to prevent the spreading of the disease, antigen heartworm tests are useful for epidemiological studies for detecting infected animals, as well as for human contamination.

## Acknowledgments

The authors thank Daniel Marhic and Abdelghani Benkhelil for their excellent collaboration.

## References

- [1] J. W. McCall, C. Genchi, L. H. Kramer, J. Guerrero, and L. Venco, "Heartworm disease in animals and humans," *Advances in Parasitology*, vol. 66, pp. 193–285, 2008.

- [2] European Scientific Counsel Companion Animal Parasites, "Worm Control in Dogs and Cats," Guideline No 1, December 2007.
- [3] F. Beugnet, S. Bima-Blum, and L. Chardonnet, "Epidemiologic study of dog dirofilariosis in New-Caledonia. Choice of a diagnosis methodology," *Revue de Médecine Vétérinaire*, vol. 144, no. 11, pp. 891–897, 1993.
- [4] American Heartworm Society, "Guidelines for the diagnosis, prevention, and management of heartworm (*Dirofilaria immitis*) infection in dogs," in *Proceedings of the Triennial Symposium of the American Heartworm Society, New Research and Additional Clinical Experience*, 2007.
- [5] C. H. Courtney and J. A. Cornell, "Evaluation of heartworm immunodiagnostic tests," *Journal of the American Veterinary Medical Association*, vol. 197, no. 6, pp. 724–729, 1990.
- [6] C. Genchi, L. Venco, and M. Genchi, "Guidelines for the laboratory diagnosis of canine and feline *Dirofilaria* infections," in *Dirofilaria immitis and D. repens in Dog and Cat and Human Infections*, G. Cringoli, Ed., *Mappe Parassitologiche*, pp. 138–144, Rolando Editore, 2007.
- [7] J. W. McCall, "An update on serological diagnosis and prophylaxis of heartworm disease in dogs and cats," in *Proceedings of the American Veterinary Medical Association Convention*, Philadelphia, Pa, USA, July 2004.
- [8] C. E. Atkins, "Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens," *Journal of the American Veterinary Medical Association*, vol. 222, no. 9, pp. 1221–1223, 2003.
- [9] C. H. Courtney and Q. Y. Zeng, "Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens," *Veterinary Parasitology*, vol. 96, no. 4, pp. 317–322, 2001.
- [10] M. Martini, G. Capelli, G. Poglayen, F. Bertotti, and C. Turilli, "The validity of some haematological and elisa methods for the diagnosis of canine heartworm disease," *Veterinary Research Communications*, vol. 20, no. 4, pp. 331–339, 1996.
- [11] S. Ranjbar-Bahadori, A. Eslami, and S. Bokaic, "Evaluation of different methods for diagnosis of *Dirofilaria immitis*," *Pakistan Journal of Biological Sciences*, vol. 10, no. 11, pp. 1938–1940, 2007.
- [12] L. Roth, L. Brown, S. Brum et al., "Comparison of three diagnostic tests for *Dirofilaria immitis* in a low-incidence area," *Journal of Veterinary Diagnostic Investigation*, vol. 5, no. 4, pp. 647–648, 1993.
- [13] J. W. McCall, N. Supakorndej, A. R. Donoghue, and R. K. Turnbull, "Evaluation of the performance of canine heartworm antigen test kits licensed for use by veterinarians and canine heartworm antigen tests conducted by diagnostic laboratories," in *Recent Advances in Heartworm Disease: Symposium '01*, R. L. Seward, Ed., pp. 135–140, American Heartworm Society, Batavia, Ill, USA, 2001.
- [14] J. M. Medlock, D. Avenell, I. Barrass, and S. Leach, "Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom," *Journal of Vector Ecology*, vol. 31, no. 2, pp. 292–304, 2006.
- [15] D. Vezzani, D. F. Eiras, and C. Wisnivesky, "Dirofilariosis in Argentina: historical review and first report of *Dirofilaria immitis* in a natural mosquito population," *Veterinary Parasitology*, vol. 136, no. 3–4, pp. 259–273, 2006.
- [16] A. C. Y. Lee, S. P. Montgomery, J. H. Theis, B. L. Blagburn, and M. L. Eberhard, "Public health issues concerning the widespread distribution of canine heartworm disease," *Trends in Parasitology*, vol. 26, no. 4, pp. 168–173, 2010.

- [17] D. Monchy, H. Levenes, H. Guegan, C. Poey, and D. Dubour-dieu, "Pulmonary dirofilariosis," *Medecine Tropicale*, vol. 53, no. 3, pp. 367–371, 1993.
- [18] C. T. Nelson, J. W. McCall, S. B. Rubin et al., "2005 Guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in cats," *Veterinary Parasitology*, vol. 133, no. 2-3, pp. 267–275, 2005.